FINAL REPORT

Microbiological Sampling Report

for

National Oceanic & Atmospheric Administration

Samplings Conducted on the Fifteenth Floor
of Building SSMC-3
on April 4, 2000

Interagency Agreement #: D8H00CO31200
Task: 9903

September 6, 2000

Prepared by

US Public Health Service

Division of Federal Occupational Health

Bethesda Central Office

Executive Summary

At the request of the National Oceanic & Atmospheric Administration (NOAA), Federal Occupational Health (FOH) conducted microbiological sampling in cubicles 15242, 15345, and 15432 of Building SSMC-3, located at 1315 East-West Highway, Silver Spring, Maryland. Sampling was conducted on April 4, 2000. Air (both Andersenâ and Zefonâ), swab, contact plate, and vacuum dust samples were collected from these cubicles and an indoor reference cubicle 15302. Air samples were also collected from outdoors.

Findings are as follows:

· Indoor airborne fungal and spore levels were much lower than those of outdoors.

· Very low fungal burden was detected from swab samples collected from surfaces of supply diffusers and return troughers in light fixture.

· Fungal levels in plenum dust of these cubicles were at 10^4 – 10^5 CFU/g of fine dust levels.

· Fungal levels in carpet and furniture dust of these cubicles were at 10^3 - 10^4 CFU/g levels.

INTRODUCTION

At the request of the National Oceanic & Atmospheric Administration (NOAA), Federal Occupational Health (FOH) conducted microbiological samplings in cubicles 15242, 15345, and 15432 of Building SSMC-3, located at 1315 East-West Highway, Silver Spring, Maryland. Sampling was conducted on April 4, 2000. Air (both Andersenâ and Zefonâ), swab, contact plate, and vacuum dust samples were collected from these cubicles and an indoor reference cubicle 15302. Air samples were also collected from outdoors.

EVALUATION METHODOLOGY

Air Samples

Various types of samples were collected from these cubicles on April 4, 2000. Two types of air samples were collected from each cubicle: (1) culturable method using Andersenâ N-6 samplers at a flow rate of 28.3 L/min, and (2) non-culturable method using Zefonâ Air-O-Cell cassettes at a flow rate of 15 L/min. Indoor Andersenâ air samples were collected for 3 minutes and outdoor samples were collected for both one and three minutes. Two percent (2 %) malt extract agar (MEA) and cellulose Czapek agar (CCA) was used to recover general fungi and cellulose-loving fungi, respectively. Non-culturable air samples were collected at the aforementioned sampling locations. Indoor samples were collected for ten minutes and outdoor samples were collected for both five and ten minutes. Outdoor air samples were collected near the entrance of the building. Temperature and relative humidity measurements were collected from each air sampling location by a battery operated, direct readout Hygroskopâ meter.

Swab Samples

Swab samples were collected from surfaces of each supply diffusers and return troughers in each cubicle. They were collected by wiping a known area of surface with a sterile cotton swab (Culturetteâ) wetted with holding media. Approximately 5 in^2 area was wiped for return trougher and 4 in^2 for supply diffusers. The swab was then placed directly into its holder. Each holder was labeled with an identifiable number. A total of 13 swab samples were collected from these cubicles.

Contact Plate Samples

To determine fungal burden on horizontal surfaces of these cubicles, three to five contact plate samples were collected from each cubicle. Sampling was conducted by pressing the MEA-filled Rodacâ plate against the surface of interest for five seconds. A total of 15 contact plate samples were collected.

Vacuum Dust Samples

Dust accumulated on carpeting, chairs and fabric system furniture, and the plenum were collected with a High Efficiency Particulate Air (HEPA) vacuum attached with a special “sock” device. For each carpet sample, a 3-ft by 3-ft area was vacuumed for at least five minutes. Total surface areas of 9 ft^2 were vacuumed from system furniture and chairs, and composite as one sample. Dust accumulated above the ceiling plenum was also vacuumed and composite as one sample. One carpet sample, one composite furniture sample, and one composite plenum sample were collected from each cubicle.

All samples collected were sent for next morning delivery to FOH’s Environmental Microbiology Laboratory (EML) in Philadelphia, Pennsylvania for analysis.

Laboratory Procedures

Upon receipt, all Andersenâ air and contact plate samples were incubated in a 25˚C incubator. Each swab sample was suspended in sterile distilled water, diluted serially, and inoculated onto agar plates. Both MEA and CCA were used for retrieving fungi. At least three dilution series were used for each sample. Each vacuum dust sample was sieved through a 250 mm sieve. The fine dust (< 250 mm) retrieved was then weighed and followed the dilution plating for fungal analysis.

All plates were incubated in a 25˚C incubator. They were examined every other day for up to 10 days to ensure the full recovery of fungi. Fungal identification was based on colony morphology, spores and conidia formation. Total fungal colonies formed on each MEA plate and *Stachybotrys chartarum* on CCA plates were counted and recorded. Fungal levels in samples were presented as colony forming units (CFUs) per measuring unit. For example, CFU/m^3 for Andersenâ air samples, CFU/in^2 for swab samples, CFU/plate for contact plate samples, and CFU/g of fine dust for vacuum dust samples.

All Zefonâ cassette samples were analyzed by the Environmental Microbiology Laboratory in Escondido, California for direct microscopic examination. Fungal spores were identified and their airborne levels were presented as spores/m^3.

RESULTS AND DISCUSSION

Temperature and Relative Humidity

Indoor temperature and relative humidity measurements ranged from 74.6°F to 75.6°F, and 34.8% – 35.3%, respectively (Table 1). Outdoors temperature reading was lower, but with a slightly higher relative humidity.

Microbiological Analyses Results

All laboratory analytical results from FOH's EML are presented in a laboratory report #NOAA-00-40R (Attachment A). Results from microscopic examination of Zefonâ cassette samples are presented in Attachment B.

Air Samples

Andersen Results

Indoor airborne fungal levels were much lower than those of outdoors (Table 1). Basidiomycetes dominated outdoor

fungal flora, followed by *Cladosporium*, *Alternaria*, and *Penicillium*. Fungi detected indoors were similar to those of outdoors. *Stachybotrys chartarum* was not detected from these samples.

Zefon Results

Indoor fungal spore levels were much lower than those of outdoors (Table 1). A more diverse fungal flora was detected from outdoors, with Basidiospores and Ascospores as the predominant fungal spores. Other fungal spores detected from outdoors were *Cladosporium*, *Penicillium/Aspergillus* types, *Aureobasidium*, *Epicoccum*, and *Pithomyces*. *Stachybotrys chartarum* was not detected from any sample collected.

Table 1. Temperature and relative humidity measurements and airborne fungal levels at different cubicles of the 15th floor in SSMC-3 on April 4, 2000.

Cubicles / Parameters	15242	15302 reference	15345	15432	Outdoors
Temperature (°F)	74.6	75.6	75.3	NA#	49.3
Relative Humidity (%)	35.3	34.8	35.3	NA	45.3
Airborne Fungal Levels (CFU/m^3)	106	71	47	35	459* 601
Total Fungal Spores ($Spores/m^3$)	20	20	40	< 7	1,234* 1,120

Data not available. * Two samples were collected from outdoors.

Swab Samples

Most (9 out of 13) samples collected from surfaces of supply diffusers and return troughers in light fixtures were below the detection limits (BDL) (3 CFU/in^2 for supply diffuser and 2 CFU/in^2 for return trougher). Other samples showed low fungal levels (2 CFU/in^2 to 4 CFU/in^2). *Aspergillus* was the predominant fungal genus recovered from these samples. *Stachybotrys chartarum* (2 CFU/in^2) was recovered from MEA plate of one sample collected surfaces of a return trougher in an unnumbered space located opposite of cubicle 15432 (sample #A38).

Contact Plate Samples

In general, fungal levels on horizontal surfaces were low, ranged from 1 CFU/plate to 9 CFU/plate. *Penicillium* was the predominant fungal genus recovered, followed by *Cladosporium* and Basidiomycetes. The highest fungal level (42 CFU/plate) was recovered from top surface of file cabinet in cubicle 15242 (sample #CP37) with *Penicillium* as the predominant fungal genus followed by *Aspergillus.*

Table 2. Fungal levels (CFU/plate) on horizontal surfaces of different cubicles at the 15th floor of SSMC-3, by contact

plate sampling collected on April 4, 2000.

Cubicles	**15242**	**15302** reference	**15345**	**15432**
Minimum – Maximum (CFU/plate)	1 – 42	4 – 5	1 – 5	1 – 9
Total Sample Number	3	3	5	4

Vacuum Dust Samples

Diverse fungal genera, such as *Alternaria, Aspergillus, Aureobasidium, Cladosporium, Epicoccum, Mucor, Paecilomyces, Penicillium, Pithomyces, Trichoderma,* Ascomycetes, and Basidiomycetes were recovered from these dust samples.

Plenum Dust

Fungal levels in the fine dust collected from the plenum were at $10^4 – 10^5$ CFU/g of fine dust levels (Table 3). *Cladosporium* and *Penicillium* were the predominant fungal genera detected from these samples. *Stachybotrys chartarum* was detected from cubicles 15242, 15432, and indoor reference cubicle 15302.

Carpet and Furniture Dust

Fungal levels in the fine dust in carpet and furniture of these cubicles were at the levels of 10^3 - 10^4 CFU/g of fine dust (Table 3). Predominant fungi detected were *Cladosporium, Alternaria,* and *Aureobasidium. Stachybotrys chartarum* was not detected from carpet dust but was detected on furniture dust collected from cubicles 15302 and 15345 (Table 3).

Table 3. Total fungal levels (CFU/g of fine dust) in fine dust collected from carpet, plenum, and furniture of cubicles 15242, 15302, 15345, and 15432 of SSMC-3, by vacuum dust sampling, collected on April 4, 2000.

Cubicles	**15242**	**15302** reference	**15345**	**15432**
Plenum (CFU/g of fine dust)	208,000 (+*)	92,000 (+)	92,000 (-)	79,208 (+)
Carpet (CFU/g of fine dust)	7,129 (-)	7,200 (-)	4,400 (-)	52,000 (-)
Furniture (CFU/g of fine dust)	26,087 (-)	5,652 (+)	23,301 (+)	3,774 (-)

*+: *Stachybotrys chartarum* was detected on MEA and/or CCA plates.

-: *Stachybotrys chartarum* was not detected on MEA and CCA plates.

CONCLUSIONS

· Indoor airborne fungal and spore levels were much lower than those of outdoors.

· Very low fungal burden was detected from swab samples collected from surfaces of supply diffusers and return troughers in light fixture.

· Fungal levels in plenum dust of these cubicles were at $10^4 – 10^5$ CFU/g of fine dust levels.

· Fungal levels in carpet and furniture dust of these cubicles were at 10^3 - 10^4 CFU/g levels.

RECOMMENDATIONS

· Conduct a thorough cleaning of these cubicles as follows:

· Wet wiping on surfaces of supply diffusers and return troughers.

· HEPA vacuuming of porous materials.

· Wet wiping on hard surfaces.

· Conduct any above ceiling plenum work after hour. Thoroughly HEPA vacuum the surrounding areas afterwards.

· Implement an emergency water intrusion protocol for this building to adequately manage any unexpected water intrusion in order to prevent fungal proliferation.

ATTACHMENT A

Microbiological laboratory report for samples collected
from fifteenth floor of SSMC-3, on April 4, 2000.

ATTACHMENT B

Results from microscopic examination of Zefon air samples collected
from fifteenth floor of SSMC-3, on April 4, 2000.

USPHS DFOH ENVIRONMENTAL MICROBIOLOGY LABORATORY, PHILADELPHIA, PA

LABORATORY REPORT #NOAA-00-40R

Client agency: National Oceanic and Atmospheric Administration, Silver Spring, MD

POIS#/task #: D8H00CO31200 / 9903

Sampling date: 4/4/00

Dates of inoculation: 4/4/00 (air and contact plate), 4/5/00 (wipes), and 4/10/00 (dust)

General location: SSMC-3, Silver Spring, MD

Specific location: 15th floor

Sampling techniques: Air (Andersen N-6 sampler), contact plate, wipe, and vacuum dust samplings

Medium used: Malt extract agar (MEA) and Cellulose Czapek agar (CCA) for fungi

Samples submitted by: J. Sobelman

Date characterization completed: 4/20/00

(A) Air samples on MEA and CCA plates

Sample ID	Sampling Location	Air Volume (L)	Fungi on MEA @ 25º C	Presence of *Stachybotrys chartarum**** on CCA @ 25º C
B6	Room 15432 and opposite cube	84.9	1. *Penicillium* (1*) 2. Basidiomycetes (2) CFU/m^3 = 35	No
B7	Room 15242	84.9	1. *Cladosporium* (2) 2. Basidiomycetes (7) CFU/m^3 = 106	No
B8	Room 15345	84.9	1. Basidiomycetes (4) CFU/m^3 = 47	No
B9	Room 15302	84.9	1. *Cladosporium* (2) 2. *Penicillium* (1) 3. Basidiomycetes (3) CFU/m^3 = 71	No

Sample ID	Sampling Location	Air Volume (L)	Fungi on MEA @ 25º C	Presence of *Stachybotrys chartarum**** on CCA @ 25º C
B10	Outside	84.9	1. *Cladosporium* (18) 2. *Alternaria* (2) 3. *Penicillium* (1) 4. Basidiomycetes (18) CFU/m^3 = 459	No
B11	Outside	28.3	1. *Cladosporium* (5) 2. Basidiomycetes (12) CFU/m^3 = 601	No
SB	**Ship blank**	NA#	No fungal growth	No

(B) Contact plate samples on MEA plates

Sample ID	Sampling Location	Fungi detected on MEA @ 25°C
CP31	Room 15432, top of desk	1. *Penicillium* (1) **CFU/plate = 1**
CP32	Room 15432, shelf	1. *Penicillium* (4) 2. *Cladosporium* (2) 3. *Aspergillus sp.* (1) **CFU/plate = 7**
CP33	Room 15432, top of system furniture	1. *Penicillium* (6) 2. *Cladosporium* (2) 3. *Aspergillus sp.* (1) **CFU/plate = 9**

Sample ID	Sampling Location	Fungi detected on MEA @ 25°C

CP34	Room 15432, top of file cabinet	1. *Cladosporium* (3) 2. *Penicillium* (2) 3. *Aspergillus niger*** (1) 4. *Aureobasidium* (1) 5. *Epicoccum* (1) 6. *Paecilomyces* (1) **CFU/plate = 9**
CP35	Room 15242, top of desk	1. *Penicillium* (1) **CFU/plate = 1**
CP36	Room 15242, top of system furniture	1. *Penicillium* (5) 2. *Paecilomyces* (1) 3. *Pithomyces* (1) **CFU/plate = 7**
CP37	Room 15242, top of file cabinet	1. *Penicillium* (22) 2. *Aspergillus niger*** (7) 3. *Cladosporium* (7) 4. *Alternaria* (3) 5. *Aspergillus sp.* (3) **CFU/plate = 42**
CP38	Room 15345, top of desk	1. *Penicillium* (1) 2. Basidiomycetes (1) **CFU/plate = 2**
CP39	Room 15345, window ledge	1. *Cladosporium* (1) **CFU/plate = 1**
CP40	Room 15345, bookshelf	1. *Cladosporium* (1) 2. Basidiomycetes (2) **CFU/plate = 3**
CP41	Room 15345, top of file cabinet	1. Basidiomycetes (1) **CFU/plate = 1**

Sample ID	**Sampling Location**	**Fungi detected on MEA @ 25°C**

CP42	Room 15345, table in front of window	1. *Penicillium* (3) 2. *Alternaria* (1) 3. *Cladosporium* (1) **CFU/plate = 5**
CP43	Room 15302, top of desk	1. *Penicillium* (3) 2. *Alternaria* (1) **CFU/plate = 4**
CP44	Room 15302, top of system furniture	1. *Penicillium* (2) 2. *Cladosporium* (1) 3. Basidiomycetes (1) **CFU/plate = 4**
CP45	Room 15302, bookshelf	1. *Penicillium* (3) 2. *Cladosporium* (1) 3. Basidiomycetes (1) **CFU/plate = 5**

(C) Wipe samples on MEA and CCA plates

Sample ID	**Sampling Location**	**Area (in^2)**	**Dilution factor**	**Fungi on MEA @ 25°C**	**Presence of *Stachybotrys chartarum**** on CCA @ 25°C**
A27	Room 15242, supply	4	10X-MEA 10X-CCA	No fungal growth CFU/in^2 < 3	No
A28	Room 15242, return	5	10X-MEA 10X-CCA	1. *Aspergillus sp.* (1) CFU/in^2 = 2	No

Sample ID	**Sampling Location**	**Area (in^2)**	**Dilution factor**	**Fungi on MEA @ 25°C**	**Presence of *Stachybotrys chartarum**** on CCA @ 25°C**
A29	Room 15345, supply	4	10X-MEA 10X-CCA	1. *Aspergillus sp.* (1) CFU/in^2 = 3	No
A30	Room 15345, supply	4	10X-MEA 10X-CCA	No fungal growth CFU/in^2 < 3	No

A31	Room 15345, return	5	10X-MEA 10X-CCA	No fungal growth CFU/in^2 < 2	No
A32	Room 15345, return	5	10X-MEA 10X-CCA	No fungal growth CFU/in^2 < 2	No
A33	Room 15302, return	5	10X-MEA 10X-CCA	No fungal growth CFU/in^2 < 2	No
A34	Room 15302, supply	4	10X-MEA 10X-CCA	No fungal growth CFU/in^2 < 3	No
A35	Room 15432, supply	4	10X-MEA 10X-CCA	No fungal growth CFU/in^2 < 3	No
A36	Room 15432, return	5	10X-MEA 10X-CCA	1. *Aspergillus niger*** (1) CFU/in^2 = 2	No
A37	Unnumbered space opposite room 15432, supply	4	10X-MEA 10X-CCA	No fungal growth CFU/in^2 < 3	No
A38	Unnumbered space opposite room 15432, return	5	10X-MEA 10X-CCA	1. *Stachybotrys chartarum**** (1) 2. *Trichoderma* (1) CFU/in^2 = 4	No
A39	Unnumbered space opposite room 15432, return	5	10X-MEA 10X-CCA	No fungal growth CFU/in^2 < 2	No

(D) Dust samples on MEA and CCA plates

Sample ID	**Sampling Location**	**Weight (g)**	**Dilution factor**	**Fungi on MEA @ 25°C**	**Presence of *Stachybotrys chartarum**** on CCA @ 25°C**
V015	Room 15432, furniture	0.053##	40X-MEA 10X-CCA	1. *Alternaria* (4) 2. *Cladosporium* (3) 3. *Epicoccum* (1) 4. *Paecilomyces* (1) 5. *Trichoderma* (1) **CFU/g = 3,774**	No

V016	Room 15432, carpet	0.100	400X-MEA 10X-CCA	1. *Mucor* (6) 2. *Aspergillus niger*** (2) 3. *Penicillium* (2) 4. *Aspergillus sp.* (1) 5. *Aureobasidium* (1) 6. *Trichoderma* (1) **CFU/g = 5.2 x 10^4**	No
V017	Room 15432, ceiling plenum	0.101	400X-MEA 10X-CCA	1. *Alternaria* (12) 2. *Aspergillus niger*** (3) 3. *Aspergillus flavus**** (1) 4. *Aspergillus sp.* (1) 5. *Epicoccum* (1) 6. *Penicillium* (1) 7. *Trichoderma* (1) **CFU/g = 7.9 x 10^4**	Yes (26) CFU/g = 2,574
V018	Room 15242, furniture	0.046##	400X-MEA 10X-CCA	1. *Alternaria* (3) 2. *Epicoccum* (2) 3. *Rhizopus* (1) **CFU/g = 2.6 x 10^4**	No

Sample ID	**Sampling Location**	**Weight (g)**	**Dilution factor**	**Fungi on MEA @ 25°C**	**Presence of *Stachybotrys chartarum**** on CCA @ 25°C**
V019	Room 15242, carpet	0.101	40X-MEA 10X-CCA	1. *Penicillium* (9) 2. *Cladosporium* (4) 3. *Aureobasidium* (2) 4. *Paecilomyces* (2) 5. *Aspergillus niger*** (1) **CFU/g = 7,129**	No

V020	Room 15242, ceiling plenum	0.100	400X-MEA 10X-CCA	1. *Penicillium* (45) 2. *Aspergillus niger*** (2) 3. *Alternaria* (1) 4. *Aspergillus fumigatus*** (1) 5. *Aspergillus sp.* (1) 6. *Cladosporium* (1) 7. *Paecilomyces* (1) **CFU/g = 2.1 x 10^5**	Yes (4) CFU/g = 400
V021	Room 15345, furniture	0.103##	400X-MEA 10X-CCA	1. *Alternaria* (4) 2. *Cladosporium* (3) 3. *Epicoccum* (3) 4. *Aureobasidium* (1) 5. Ascomycetes (1) **CFU/g = 2.3 x 10^4**	Yes (2) CFU/g = 97
V022	Room 15345, carpet	0.100	40X-MEA 10X-CCA	1. *Cladosporium* (4) 2. *Aureobasidium* (3) 3. *Nigrospora* (2) 4. *Alternaria* (1) 5. *Epicoccum* (1) **CFU/g = 4,400**	No

Sample ID	**Sampling Location**	**Weight (g)**	**Dilution factor**	**Fungi on MEA @ 25°C**	**Presence of *Stachybotrys chartarum**** on CCA @ 25°C**
V023	Room 15345, ceiling plenum	0.100	400X-MEA 10X-CCA	1. *Aureobasidium* (9) 2. *Alternaria* (7) 3. *Epicoccum* (6) 4. *Cladosporium* (1) **CFU/g = 9.2 x 10^4**	No

V024	Room 15302, furniture	0.092##	40X-MEA 10X-CCA	1. *Cladosporium* (9) 2. *Penicillium* (8) 3. *Aspergillus niger*** (3) 4. *Alternaria* (2) 5. *Aspergillus sp.* (1) 6. *Aspergillus versicolor**** (1) 7. *Epicoccum* (1) 8. Basidiomycetes (1) **CFU/g = 5,652**	Yes (9) CFU/g = 489
V025	Room 15302, carpet	0.100	40X-MEA 10X-CCA	1. *Cladosporium* (5) 2. *Alternaria* (3) 3. *Epicoccum* (3) 4. *Aureobasidium* (2) 5. Ascomycetes (5) **CFU/g = 7,200**	No

Sample ID	**Sampling Location**	**Weight (g)**	**Dilution factor**	**Fungi on MEA @ 25°C**	**Presence of *Stachybotrys chartarum**** on CCA @ 25°C**
V026	Room 15302, ceiling plenum	0.100	400X-MEA 40X-CCA	1. *Penicillium* (13) 2. *Cladosporium* (3) 3. *Alternaria* (2) 4. *Aspergillus sp.* (2) 5. *Aspergillus niger*** (1) 6. Basidiomycetes (2) **CFU/g = 9.2 x 10^4**	Yes (1) CFU/g = 400

* Colony counts.

** Opportunistic fungi.

*** Toxigenic fungi.

Not applicable.

5ml of sterilized distilled water were added instead of 10ml.

Characterization completed by: _____

Ling-Ling Hung, Ph.D. Microbiologist

Quality control checked by: _____ (initials)